Impact of Vascular Endothelial Growth Factor on Skin Graft Survival in Irradiated Rats

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Objective: To evaluate the effect of vascular endothelial growth factor (VEGF) on full-thickness skin graft (FTSG) survival on irradiated tissue as a model of wound healing in ischemic conditions.

Design: Twenty-four Sprague-Dawley rats underwent 30 Gy of irradiation to their left dorsum (10 Gy/d). After 4 weeks of recovery, 3-cm FTSGs were harvested from the healthy contralateral dorsum and placed onto irradiated recipient beds. Before grafting, recipient beds were delivered subfascial injections of either VEGF protein (5 µg) or physiologic saline. Graft failure (more than 10% necrosis) and graft microvascular density were compared between groups.

Results: Seven of the 11 FTSGs from saline-treated irradiated beds (64%) failed, whereas the failure rate for grafts treated with VEGF was 23% (3 of 13) (P = .048). Mean microvascular density was not different between groups.

Conclusion: Exogenously administered VEGF may improve the outcome of FTSGs on irradiated tissue beds.

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Schismic and unhealthy wound beds provide a challenge to the reconstructive surgeon. The survival of skin grafts at these sites is variable and may influence the decision to use alternative reconstructive options when skin grafts are a logical choice. In particular, the negative effects of radiation on wound healing limit the viability of skin grafts placed onto the recipient beds. Endarteritis obliterans, excessive fibrosis, and impaired cellular turnover contribute to reduced vascularity, blood flow, and nutrition in irradiated tissue. This severe effect of radiation on soft-tissue health can thereby provide a valuable tool for examining factors involved in the survival of skin grafts on compromised wounds. Specifically, molecular agents that enhance the vascularity of ischemic recipient sites should improve the success rate of skin grafts and be reliably illustrated in a model of radiation injury.

Vascular endothelial growth factor (VEGF), a potent endothelial cell mitogen and permeability factor, has been shown to stimulate angiogenesis, vascular remodeling, and new tissue growth. Many investigators have exploited the use of exogenous VEGF in models of soft-tissue reconstruction with evidence of improved survival of skin flaps. Simularly, our group has previously shown improved viability of full-thickness skin grafts (FTSGs) after application of recombinant VEGF to their recipient beds. However, the impact of exogenous VEGF on FTSG acceptance in recipient beds with prior radiation exposure has not been examined, to our knowledge. In this study, we evaluated the impact of recombinant VEGF on FTSG survival in a rat model of irradiated recipient beds.

METHODS

Twenty-four adult Sprague-Dawley rats weighing 250 to 300 g were used in this study after approval of the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences was obtained. Standard living conditions for the rats were provided by animal laboratory personnel with use of day-to-night light cycling and standard rat chow. All surgical procedures were conducted under sterile conditions with minimal variation in performance.

IRRADIATION PROCEDURE

Animals were anesthetized by isoflurane inhalation. A circular area with a diameter of 3 cm marked on the left dorsum of each rat was exposed to a total radiation dose of 30 Gy (to convert to rad, multiply by 100). All animals received a daily dose of 10 Gy for 3 consecutive days under gen-
eral anesthesia. Irradiation was performed with an x-ray machine (Isovolt 320; Seifert X-Ray Corp, Fairview Village, Pennsylvania). Irradiation characteristics were 250 kV, 15 mA, with 3-mm aluminum as added filtration. The resulting half-value layer was 0.85 mm of copper, at a dose rate of 4.49 Gy/min.

**SURGERY**

The animals were allowed to recover for 4 weeks after their last radiation dose. Animals were anesthetized by means of intraperitoneal injection of pentobarbital sodium (40 mg/kg) and placed in prone position on a standard surgical platform with the dorsum exposed. The entire dorsum was shaved, prepared, and draped by means of standard sterile surgical technique. The previously irradiated area was again marked with a 3-cm-diameter circle on the left dorsum. A circle of the same size was outlined on the right dorsum as the donor site for the FTSG (Figure 1A). Perioperative antibiotics (cefazolin sodium, 30 mg/kg) were administered and incisions were made along the previously outlined marks. Incisions were carried below the panniculus carnosus muscle to the superficial fascia layer. Once the grafts were harvested, the panniculus carnosus muscle was dissected from the overlying dermis. This was performed to simulate human FTSGs where subcutaneous fat is removed before graft placement.

Animals were randomized to receive subfascial injections of either recombinant VEGF protein (1.0 mL of 5 µg/mL; R&D Systems, Minneapolis, Minnesota; 13 rats) or physiologic saline (1.0 mL; 11 rats) in the irradiated recipient bed in the left dorsum of each animal. The number of animals in each group was slightly unbalanced owing to erroneous injection of VEGF instead of saline in 1 animal. The VEGF or saline was distributed evenly among 10 equally spaced injections of 0.1 mL using a 30-gauge needle and a tuberculin syringe (Figure 1B). The skin grafts taken from the nonirradiated right side of the dorsum were immediately placed on the VEGF- or saline-treated and previously irradiated recipient beds and secured by means of 3.0 polyglactin 910 sutures (Figure 1C).

On postoperative days 14 to 18, the animals were anesthetized again and the skin grafts were harvested. The rats were humanely killed with a 200-mg/kg intraperitoneal injection of pentobarbital sodium. All except 1 FTSG underwent 100% epidermolysis (Figure 2A). As performed in human skin grafts, the epidermal scab was removed before evaluation of the underlying dermal graft for healthy and necrotic areas.

**PLANIMETRY**

As previously described, the areas of graft necrosis were identified and measured by planimetry.8,11,12 After removal of the layer of epidermolysis, transparency film was used to mark the entire surface of the skin grafts as well as their necrotic areas by an experienced surgeon (E.V.) blinded to the treatment groups. Regions of white dermis with punctate bleeding or cap-
illaries were considered healthy. Areas of dark gray/brown discoloration or complete tissue loss exposing the underlying facial compartment were considered necrotic and were marked as such (Figure 2B and C).

The transparencies were then digitally scanned for planimetry, and a percentage of the total graft area was calculated by computer software (Image Pro Plus, Silver Springs, Maryland). To simulate clinical practices with a high threshold for success measures, grafts were considered to be successful if necrosis was equal to or less than 10% of the entire graft surface area. Any graft necrosis consisting of more than 10% of the surface area of the graft was considered to represent failure. Graft success rates were then compared between control (saline-treated) and VEGF-treated groups. After planimetric analysis, the grafts were harvested and placed in formalin solution for histologic examination and immunostaining.

**MICROVASCULAR DENSITY**

Standard hematoxylin-eosin staining was performed for microvascular density analysis. The pathologist (C.-Y.F.), blinded to the treatments, measured microvascular density for each FTSG. Slides were initially scanned under low-power microscopy (×100) to identify prominent vascular areas. Representative regions were then examined at ×400 magnification and individual vessels were counted in 3 consecutive high-power fields. Mean vascular count for each graft was then calculated.

**STATISTICAL ANALYSIS**

Graft success rates were compared between groups by χ² analysis. Mean vessel counts were compared between VEGF-treated and saline-treated groups by a 2-tailed t test. The statistical significance was determined at P ≤ .05. All statistical tests were conducted with Microsoft Excel 2002 (Microsoft Corp, Redmond, Washington).

**RESULTS**

Nearly 100% epidermolysis was discovered on each graft of both the saline- and VEGF-treated irradiated beds. The epidermis was essentially a scab that was removed before examination of the healthy underlying dermal bed, as performed in healing human FTSGs. Twenty-three percent (3 of 13) of FTSGs from the VEGF-treated irradiated beds had evidence of graft failure (>10% necrosis), while the failure rate for grafts from saline-treated recipient beds was 64% (7 of 11) (P = .048). No significant difference was observed in mean (SD) vascular density between recombinant VEGF-treated (13.9 ± 1.6) and saline-treated (15.9 ± 1.7) skin grafts (P = .42).

**COMMENT**

Reconstructive surgery often entails using grafts, which are avascular in nature. Skin, fat, dermis, fascia, cartilage, and bone grafts all have an area of application in reconstructive surgery. However, viability of these graft materials is solely dependent on their revascularization, and this process is often compromised in certain conditions delaying wound healing, such as previous radiation exposure. Excessive fibrosis, endothelial cell damage, and reduced cellular turnover follow radiation injury. Vascular occlusion secondary to endarteritis obliterations and reduced vascular regulation further causes ischemia and reduced health of irradiated tissue. Thus, reconstructive efforts in tissues with previous radiation exposure can be challenging, especially with the use of avascular tissue such as grafts.

Vascular endothelial growth factor is a homodimeric 43-kDa protein with known proangiogenic, vascular permeability, and chemotactic properties implicated in the complex cascade of wound injury and repair. Four splice variants of VEGF messenger RNA (VEGF 121, 165, 184, and 209) with a high degree of species protein homology have been described (rat VEGF 164 vs human VEGF 165). This has allowed the use of animal studies to investigate the potential role of VEGF in human physiology.

The exogenous administration of VEGF has been shown to improve skin flap survival. In a previous model our group investigated the impact of VEGF on FTSGs in the Sprague-Dawley rat and showed improved dermal survival after administration of VEGF into the recipient beds. The validity of this model has been supported with a more recently published article describing similar methods. The current study examines the impact of exogenous VEGF on FTSGs placed on recipient beds compromised by radiation exposure. We have found that overall graft success rate, which had been predefined as equal to or less than 10% loss, was significantly increased in grafts with VEGF-treated recipient beds when compared with the grafts placed on saline-treated recipient beds (P = .048).

Despite the significant improvement in graft success rates, the microvascular density between grafts placed in VEGF- and saline-treated recipient beds did not show a statistically significant difference. This may be surprising because of the well-known angiogenic properties of VEGF. However, it has been previously demonstrated that VEGF may not have an effect on the mean number of vessels in transplanted tissue models despite improved survival after exposure to exogenous VEGF. These results have been attributed to an increase in newly formed vessels in the recipient bed but not in the transplanted tissue. Similarly, increased blood flow in VEGF-exposed capillaries along with rapid vascularization in the transplanted tissue without a change in the actual number of vessels by VEGF administration may account for this discrepancy.

The translational potential of this study involves the development of immediate, readily available, and relatively simple reconstructive modalities such as skin grafting for compromised wound beds and, less likely, injuries resulting from a massive nuclear catastrophe. In the latter scenario, donor sites for skin grafts may also have been exposed to radiation, and investigation into the fate of skin grafts removed from irradiated donor sites would be valuable. The use of VEGF-treated split-thickness skin grafts in irradiated recipient beds also needs examination. Nonetheless, we have demonstrated improved survival of FTSGs with exogenous VEGF after radiation exposure in the recipient beds in a rat model. This study, as well as other animal models of improved survival of prefabricated irradiated flaps with VEGF, indicates that this important molecule may be a potential means of improving graft and flap survival in patients with compromised recipient beds such as irradiated ones.
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REFERENCES


